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Thermogenesis of certain hay-inhabiting fungi

James William Harrison
Iowa State College

NOTE TO USERS

THERMOGENESIS OF CERTAIN HAY-INHABITING FUNGI

By

James William Harrison

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Signature was redacted for privacy.

In Charge of Major Work

Signature was redacted for privacy.

Head of Major Department

Signature was redacted for privacy.

Dean of Graduate College

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TABLE OF CONTENTS

Table of Contents Concluded

INTRODUCTION

Microbial thermogenesis by fungi had been a subject of controversy when living substrates were concerned until the work of Miehe (18) in Germany, Norman (19) in England and Gilman and Barron (7) in this country established conclusively that certain fungi were capable of developing considerable amounts of heat when grown on hay, straw, or stored grain. The fact of thermogenesis by certain fungi having been established, the obvious questions of how far such phenomena might extend through the mycological world and the mechanism by which such heat was liberated immediately come to mind.

In order to advance our knowledge in these directions the present investigation was undertaken. A limited number of fungi were isolated from alfalfa hay, which had heated in the mow and which was in good condition; and the thermogenic power of these fungi investigated. Since this power might well be a function of growth, and particularly of respiration, according to the findings of plant physiologists, investigations into the growth rate of the above fungi and the relation of their thermogenesis to CO_2 production were conducted in an attempt to gain a better insight into the mechanism of heat liberation.

Before presenting the data gathered on these two lines of investigation a brief history of the problem will present a background for the better understanding of the progress made.

HISTORICAL

The problem of rising temperatures in decomposing plant materials has been given some attention by scientific workers for many years. This problem was first investigated by chemists who thought only in terms of the very high temperatures which immediately preceded spontaneous ignition. Many theories were advanced to explain the development of this temperature which in general was attributed to the action of inorganic catalysts. No attention was paid to the role of microorganisms which were considered to be accidental invaders that benefited by, rather than caused, any heat production. Since the earliest theories were well summarized by Brown (1), it is unnecessary to repeat them here. However, the investigations directly pertinent to the present problem may be profitably discussed.

It was not until the beginning of the nineteenth century that the botanists themselves understood that heat is given off by respiring plant tissue. From this time the idea grew very slowly and not until 1830 was it tested experimentally. At this time Göppert (9) placed germinating grain in a box and measured the temperature developed. His material was not sterile; therefore, the temperatures he obtained were the result of the joint activity of the germinating grain and the adhering microorganisms.

No further contribution was made to the subject of thermo-

genesis until 1890 when the problem of heat development in germinating barley was again taken up by Cohn(2).This investigator differentiated between the temperature developed by the germinating barley itself, and that released by the activity of adhering microorganisms. In his experiments he found a rather rapid rise in temperature to about 40°C. and then a pause, followed by a second rise to 65°C. The first rise, he attributed to the activity of the respiring plant tissue and the rise from 40°C. on, to the microorganisms. He attempted to establish this fact by observing the amount of heat produced in germinating barley after he had treated it with a solution of copper sulphate. In grain thus treated he obtained less than 40°C., but on reinoculation the temperature rose rapidly to over 55°C. In further experiments with cotton he found that, if this material was sterilized by live steam, it neither fermented nor heated, but the temperature rose rapidly when inoculated with washings from old cotton waste.

The fact that microorganisms are always present in the earlier stages of heating, in organic materials, forced itself into the cognizance of workers, more and more as time passed. It was suggested that the microorganisms may themselves function in the development of the heat; and that the subject should be investigated.

In 1906 Duggeli (4) made a number of bacterial counts from heating hay. He found several types of organisms and came to the

conclusion that microorganisms are capable of generating heat in

hay to a temperature of some 70°C., and that during the process both the numbers and the dominating types change. This conclusion, arrived at incidentally, has since been checked under experimental conditions by several other investigators.

In 1907 Miehe (17) reported his findings in a series of experiments he had carried on during the three years previous. From his studies, which centered on the thermophilic bacteria, he concluded that microorganisms were able to raise the temperature of their substrate to as high as 70°C. He was able to show that hay in particular, would not heat if the moisture content was held too low for microorganisms to develop. He also stated that in dead plant tissues, microorganisms are the only agencies in the initiation of heat.

No further information was added until 1923 when Haldane and Makgill (10) arrived at similar conclusions to those of Miehe, by a different approach. They were working on the rates of oxygen consumption and the liberation of CO_2 on wetted hay at various temperatures, when they noted fluctuations which at first they were unable to account for. They subsequently found that these fluctuations were due to the activity of bacteria. These workers concluded that microbial activity proceeds concurrently with a simple oxidation of the hay itself, and the fluctuations in oxygen consumption really confirmed the bacteriological conclusions of Miehe.

The conclusions of Miehe were further confirmed by Hilderbrandt (12) in 1927, who, by using various methods of steriliza-

tion, found that sterilized hay was incapable of developing heat, and that its temperature would rise if it was inoculated with microorganisms. He also found that when the moisture content of hay was held just below the requirement for the development of the micro-flora, no heating occurred.

Simultaneously with, but independently of, the work of Hilderbrandt, studies of microbial thermogenesis were being extended to other plant products by James (13) and James, Rittger and Thom (15). These investigators, using a specially constructed apparatus, found a number of microorganisms, chiefly bacteria, to be thermogenic, when placed on corn meal, cracked corn and organic materials. Two forms of special interest among those reported were Aspergillus fumigatus, which raised the temperature of its substrate to 51.5°C., and an "unidentified mold" which developed 46.5°C. The data brought forward indicated that microorganisms played a very important role, at least in the early stages, in the heating of plant products. Associated with heating, they reported a great lossof dry matter, principally of the carbohydrates.

Whatever doubt may have existed about fungous thermogenesis, should have been dispelled in 1930, when three investigators working independently, published conclusive data specifically involving the fungi. The papers referred to were published by Miehe (18) in Germany, Norman (19) in England, and Gilman and Barron (7) at the Iowa State College. Any one of the reports alone was convincing but the three coming almost simultaneously

from widely scattered sources, and involving various organisms and substrates, established beyond question, that many fungi are able to release sufficient heat to markedly raise the temperature of the substrate upon which they were growing.

By means of a carefully planned group of experiments, Miehe was able to answer critics of his earlier work and to more firmly establish his conclusions regarding the role of microorganisms in thermogenesis. He also showed a wide difference between the amount of heat developed by germinating seeds when they had been treated to eliminate microorganisms, and that of untreated seed. By germinating sterilized sunflower seeds in a thermos flask, he observed a temperature of only 23°C., while on the other hand the temperature of untreated seed rose to 58.5°C. He then inoculated the sterile material with washings from the unsterile flasks and the temperature rose from 23 to over 44°C.

In his further studies with pure cultures of fungi on bread and other substances, he showed that Rhizopus nigricans, Mucor corymbifer, Aspergillus niger and Aspergillus fumigatus were thermogenic, when placed under suitable conditions on any substrate available to the organism for its vital activities.

Working on the biological decomposition of plant materials, Norman (19) reported a number of species of fungi as thermogenic, namely: Aspergillus terreus, A. nidulans, A. fumigatus and Trichoderma sp. These organisms were grown on sterilized straw to which was added some form of nitrogen. Their ability to raise the

temperature of the substrate was studied both in pure culture and in predetermined associations. The association of organisms was apparently either cooperative or competitive in thermogenesis. Extending this work to the amounts and types of dry matter lost, the period of maximum heat production was shown to correspond to that of the greatest loss of hemicelluloses (20).

The conclusions of Gilman and Barron (7) are largely in accord with those of Miehe. These workers, in some carefully controlled experiments, showed the rising temperatures in stored grain were due to the activity of fungi and that only a small amount of heat was developed by the germination of the grain alone. The data presented showed that in no case did the temperature of germinating seed, which had been treated to destroy the adhering micro-flora, rise above 25°C. The temperature of grain inoculated with cultures of Aspergillus fumigatus, A. flavus or A. niger rose to more than 50°C. They concluded that these fungi were very active in producing heat, when grown on moist grain, and by far the greater amount of heat produced in stored grain was due to the activity of microorganisms.

In the mycological laboratory at Iowa State College another phase of microbial thermogenesis was investigated by Gaskill (6). In his experiments, this worker grew species of fungi, of proved thermogenic ability, on corn-cob meal, in which the amounts and types of nitrogen were controlled. He found that the thermogenic ability of the organisms studied increased to a certain amount

with the increase in the amount and availability of the nitrogen present in the substrate. He further found that the fungi studied responded in the amounts of heat developed, to changes of moisture content and to the amount of aeration of the substrate.

MATERIALS AND METHODS

Isolation of Organisms

Isolations of fungi were made from alfalfa hay which had heated spontaneously to some 60°C. in an experimental storage-mow (11). When this mow was opened many yellowish green patches, which later proved to be composed of fruiting heads of Aspergillus flavus Link, were noted. A number of samples of the hay from various levels were placed immediately in sterile moist-chambers. Later these samples were divided and portions of each incubated at room temperature, at 30°C., and at 40°C.

As fruiting heads appeared during the incubation period, they were picked off with a sharp pointed, nichrome needle and streaked on hay infusion agar in petri dishes. These streaks were allowed to stand for a few days at room temperature and examined frequently. If the cultures were pure they were transferred to agar slants. It was often necessary to pick off other heads from the subcultures and place them in sterile water blanks and then make streaks of the spore suspension. Although pure colonies were rather easily obtained by this means, only four species of fungi were represented:

Aspergillus flavus Link

A. fumigatus Fres.

A. terreus Thom

Rhizopus tritici Saito

From the above substrate only the thermoduric forms of fungi were obtained. However, in the course of subsequent investigation, each of these forms proved, not only able to endure heat, but also, to raise considerably, by its own metabolic activity, the temperature of certain substrates. In addition to the thermoduric forms, there are many other fungi which normally inhabit alfalfa hay. To isolate the latter, some good quality hay was thoroughly wetted with sterile, distilled water and incubated at room temperature. As the fruiting heads of fungi appeared they were picked off and transferred to agar slants. In addition some of the hay was washed with sterile, distilled water and the washings plated. Many colonies of fungi and bacteria appeared on the plates. The fungi were transferred to agar slants and the bacteria discarded. The following species were obtained:

Aspergillus flavus Link

A. fumigatus Fres.

A. terreus Thom

A. niger Van Tieghem

A. clavatus Desm.

Penicillium oxalicum Currie and Thom

P. Humicola Oudemans

Spicaria divaricata (Thom) Gilman and Abbott

Mucor abundans Povah

Rhizopus tritici Saito

R. nigricans Ehrenberg

Cunninghamella elegans Lendner

Hormodendron nigrescens Paine

Alternaria humicola Oudemans

This group is by no means a complete list of the fungi that may be found on hay, but, rather, represents the more common inhabitants, at least under the conditions extant at the time the isolations were made.

In subsequent experiments several of these forms, although unable to survive the higher temperatures, were found to release heat in considerable quantities.

Preparation of Substrate

For identification and for the study of growth rates, Czapek's medium, prepared in the usual manner (24), was used entirely.

Alfalfa hay for the thermogenesis experiment was obtained from the Iowa State College Dairy Farm. This hay was selected because it was of good quality, and it had been chopped by machinery into short, rather uniform and convenient lengths for manipulation. Approximately sixty grams of the hay was placed in a number of glass tubes, 18" x 1¼", which were plugged at each end with cotton, and sterilized at 15 pounds pressure for one hour on each of four consecutive days. This treatment, though severe, was found to be essential to assure complete sterilization. The

moisture content was then determined. This determination was necessary in order that the proper adjustment could be made by the addition of sterile water at the time of inoculation.

Preparation of the Flasks

Ordinary commercial thermos flasks, 450 cc. capacity, were used in all experiments. These flasks were first thoroughly washed in boiling water and then kept in a 1-500 solution of $HgCl_2$ for several days. Just before using, they were rinsed several times, first with scalding water from the high pressure steam boilers, and finally with several changes of sterile, distilled water. The latter helped to cool the flasks to room temperature. The flasks were closed with rubber stoppers which had been treated with fifty per cent alcohol. It was later found by another worker in this department that the flasks could be plugged with cotton and safely sterilized in the autoclave.(6).

Packing the Flasks

The flasks were packed by removing the plug from one end of the tube of hay, inverting the tube over the mouth of a sterile flask, and pushing the hay ahead of the upper plug by means of a glass rod. The flask was immediately closed with a rubber stopper which had been treated with fifty per cent alcohol. The operation was carried on in the inoculating chamber and the usual precautions taken to prevent contamination.

The Inoculum and Inoculation

In the inoculation of the substrate, there were two problems to be considered, the even distribution of the spores and the proper adjustment of the moisture content. Large cultures of the desired organism were grown on agar slants in sixteen ounce medicine bottles. As soon as sufficient spores were produced, the slant was washed with enough sterile, distilled water to bring the contents of a flask to the desired moisture content. The spore suspension was poured into the hay and the flask was closed with either a sterile rubber stopper or a cotton plug through which passed a sterile thermometer. The inoculated flasks were then rolled for about fifteen minutes to even the distribution of the moisture and of the spores, after which they were allowed to lie on their sides for an hour and rolled at frequent intervals.

Check

A check indicates a flask loaded with sterile hay and brought to the same moisture content as the inoculated flasks. In a few cases a dry check was also used, but as it made no contribution of useful information, it was dropped from the experiment.

Methods of Observation

Method of determining growth rates.

Growth rates were determined on petri dishes containing 10 cc. of Czapek's medium streaked with three drops of a spore suspension of the organism to be observed. A sufficient number of plates were inoculated to allow one or more to be incubated at each five degree interval from 0°C. to 50°C.

As germination occurred, the developing hyphae were measured at frequent intervals with an eyepiece micrometer until the spore could no longer be seen. As a rule twenty developing hyphae were selected at random over a small area, measured, and their average length calculated. To give some index of comparison, the number of microns of growth was divided by the number of hours and the quotient recorded as the growth index.

This program was carried on in convenient series until all the organisms had been grown at each temperature interval. Although it was necessary to divide the work into small sections to facilitate handling, the conditions of the experiment were kept as nearly constant as possible.

Determination of thermogenesis.

During the study of thermogenesis two objectives were sought:

first, a measurement of the rise in temperature, and second, the relation of this rise to respiration. Therefore, two series of observations were made: one to determine temperature alone and another to obtain temperature readings simultaneously with the measurement of CO_2 production.

In the first series the flasks were closed with cotton plugs and allowed to stand on the laboratory tables subject to the temperature changes of the room. Readings were made by means of thermometers, usually at 9:00 a.m. Record was made of each flask, of the check, and of the room temperature. Observations were also made on several organisms with the flasks placed in the constant temperature bath.

The purpose of the experiments carried on in the water bath was to determine the effect of the fluctuations of room temperature on the progress of the heat curve and on the maxima. Where such additional observations were made, they were recorded. The observed differences in temperature between a series of flasks held in the constant-temperature bath and a series held at room temperature were no greater than the differences between individual members of either of the series. For the present experiment, the water-bath was considered an unnecessary refinement.

In the second series the flasks were so arranged that temperature readings could accompany the determination of carbon-dioxid evolution. For this purpose a thermocouple was passed through the rubber stopper into the mass of heating material and the

temperature read on the dial of the potentiometer directly in degrees Fahrenheit. These readings were immediately converted into degrees Centigrade and recorded. At each reading the accuracy of the potentiometer was checked against a calibrated thermometer in warm water, and if necessary, the instrument was adjusted by means of the device attached to it for this purpose.

To facilitate handling a number of flasks, a rotary switch was placed between the flasks and the potentiometer.

Determination of carbon dioxid evolution.

To determine the rate of carbon dioxid evolution, special absorption towers, a modification of those described by Emerson (5) were built. Immediately following the first daily temperature reading, the vitiated air was drawn from the flasks and passed through a n/10 solution of $Ba(OH)_2$. Aspiration was continued until, as nearly as possible, the entire air of each flask had been displaced by CO_2 free air. After allowing sufficient time for the precipitate to settle out, the collection tubes were detached and the contents rapidly filtered through a tared filter paper. After drying, the precipitate was weighed, and the CO_2 evolution in milligrams per hour calculated. This method can only be relative, but should furnish an index of the relation between CO_2 evolution and the rate of heat production.

For general construction of the apparatus see plate I.

PRESENTATION OF DATA

The data are presented for each organisms as an individual species. To facilitate comparison they are arranged in the following order for each case: first, a brief discussion of the organism; second, its growth index; third, its thermogenesis, and finally, a summarization of the findings made in this investigation and a comparison of them with those of earlier workers. (The graphic presentations have been brought together at the close of the paper).

As a group, the members of the genus Aspergillus were the more vigorous heat producers. For this reason they are discussed first.

Aspergillus flavus Link

As stated before, the original culture of A. flavus from which the subcultures used throughout the experiment were obtained, was procured from a heating mow, and had survived a temperature of some 60°C. This form complied closely to the description given in Thom and Church (23). There was some evidence that the culture was composed of several strains which did not differ sufficiently to be separated except with great difficulty. As far as can be seen, no useful point would be gained by making such a separation.

Growth index.

The growth index for A. flavus at various temperatures is given in the following table: This index was obtained by dividing the number of microns increase in length of the germ tube by the number of hours occupied in growth.

Table I. Growth index for A. flavus as expressed in μ / hrs.

Temp. °C.	1-8 hrs.	9-24 hrs.	25-48 hrs.
5	0	0	0
10	0	0	3
15	0	0	1
20	0	10	66
25	0	9	297
30	17	490	333
35	0	88	88
40	3.6	66	41
45	0	45	
50	0	0	0

At 30°C., germination took place in a few hours followed by a rapid growth which reached its peak in twenty-four hours. The rate of growth then slowed down and the lower rate was continued until the colonies interferred with each other. After six days little difference could be seen in the size of colonies grown at temperatures from 30°C. to 40°C. inclusive. At 45°C. the media dried out in twenty-four hours, therefore lack of moisture may have been the limiting factor. It would appear from the data that 30°C. was the optimum temperature though A. flavus grew well over a rather wide temperature range. Even at 10°C.

and 15°C., colonies 2 mm. in diameter with spores were produced in seven days.

When the growth rates were compared with the tables for thermogenesis it was seen that the period of rapid temperature accumulation in the inoculated flasks corresponded closely with the period of the most rapid growth of the fungus on artificial media.

Thermogenesis.

The data on thermogenesis in table II were obtained from thermometer readings of a series of flasks under the following described conditions. Five flasks were prepared; three were inoculated with spore suspensions of A. flavus and all were brought to 40 per cent moisture content. The flasks of this series were plugged with cotton and sterile thermometers passed through the plugs. In one of the flasks was a calibrated thermometer graduated to 0.1 °C. This flask, with a check, was placed in a constant temperature bath at 26°C. and observed at frequent intervals as shown in table II. The remainder of the series were allowed to stand on the laboratory tables at room temperature. During the period of observation the building was heated and the temperature, as shown by a thermograph, fluctuated only within a narrow range.

The temperature readings in table III were made by means of a thermocouple.

Table II. Thermogenesis for Aspergillus flavus given in °C.

Day	Hour	Water bath 26°C. Inoculated flask	Water bath 26°C. Check	Laboratory table Flask 1	Laboratory table Flask 2	Laboratory table Check	Room
0	2:00 p.m.	27	27	25	25	25	26
1	8:00 a.m.			28	27	24	23
	9:00	30.7	27				
	12:00 m.	31.0					
	1:00 p.m.	31.5					
	2:00	32.0					
	3:00	33.0					
	4:00	33.7					
	5:00	34.4					
	6:00	35.3					
	7:00	36.2					
	8:00	36.8					
	9:00	37.7					
	10:00	38.3					
2	8:00 a.m.	41.4	27	36	38	24	24
	12:00 m.	41.7					
	3:00 p.m.	41.7					
	5:00	41.7					
3	8:00 a.m.			40	41	25	24
	9:00	42.5	27				
	12:00 m.	43.3					
	5:00 p.m.	43.8					
4	8:00 a.m.			37	39	24	23
	9:00	38.7	27				
	12:00 m.	38.5					
	10:00 p.m.	38.0					
5	8:00 a.m.			37	38	24	24
	9:00	36.5	27				
	5:00 p.m.	37.2					
6	8:00 a.m.			38	38	24	24
	9:00	38.6	27				
7	8:00 a.m.			35	34	23	22
8	8:00 a.m.			30	31	24	24

Table III. Temperature and CO_2 production of Aspergillus flavus on hay. Forty per cent moisture content.

Day	Hour	Flask 1 Temp. °C.	Flask 1 CO_2 mgs.	Flask 2 Temp. °C.	Flask 2 CO_2 mgs.	Flask 3 Temp. °C.	Flask 3 CO_2 mgs.	Flask 4 Temp. °C.	Flask 4 CO_2 mgs.	Check °C.	Room °C.
0	9:00 a.m.					26		26		26	26
0	12:00 m.	24		24						26	26
1	8:00 a.m.	29.8	.68	30	1.0					26	25
	9:00					32	1.6	31.5	1.3	26	26
2	8:00	30.8	1.00	37	1.5					25	24
	9:00					34	2.5	33.6	2.0	26	24
3	8:00	35.0	1.9	40	2.5					25	24
	9:00					35.8	3.0	35.0	3.0	23	24
4	8:00	40.0	2.0	41	2.0					24	23
	9:00	[illegible]	[illegible]			36.6	3.0	36.6	3.0	26	26
5	8:00	41.5	2.0	42	2.5					24	25
	9:00					37.6	3.0	37.6	3.0	26	26
6	8:00	36.0	3.2	36	3.0					24	26
	9:00					38.5	3.2	38.6	3.2	24	26
7	8:00	37.0	3.0	35	2.0					25	26
	9:00					33.6	3.0	33.6	3.0	26	25
8	8:00	35.0	3.5	35	2.0					25	25
	9:00					30.0	2.0	31.0	1.5	26	25

In the check only a very small amount of CO_2 developed, so small as to be hardly measurable.

At the close of all experiments platings were made, and for all the cases given, the organism was recovered in pure culture. It was also established that no invaders were present.

It will be seen from the above data that A. flavus was able to produce considerable quantities of heat when alfalfa hay was used as a substrate. The maximum reached was 43.8°C. on the third day. This organism has been shown by Gilman and Barron (7) to be capable of producing 51.1°C. on oats and 49.4°C. on barley. Gaskill (6), with certain controlled sources and amounts of nitrogen and of moisture, was able to show a still wider range.

Examining the curve of carbon dioxid production one may see that the progress of the curve simulates, to some extent, the curve for heat production. As indicated in some later experiments, however, it is very doubtful if the carbon dioxide production could be used as an index to the amount of heating.

Aspergillus terreus Thom

The culture of A. terreus used in this experiment survived heating in the mow to some 60°C. It was also found consistently on all the moistened samples of hay, though, as a rule, its appearance was a little slower than that of A. flavus. In cultural characteristics it conformed closely to the description given by Thom and Church (23). At lower temperatures A. terreus grew very slowly, but from 25°C. to 45°C. it grew well, with

its optimum at about 35°C.

Growth index.

The microscopic study of growth could not be carried on with any degree of accuracy after the end of the forty-eight hour period due to the disappearance of the original spore and the interweaving of the hyphae. The macroscopic measurements, however, were indicative.

Table IV. Growth index for A. terreus as expressed in μ/hrs.

Temp. °C.	1-8 hrs.	9-24 hrs.	25-48 hrs.
5	0	0	0
10	0	0	0
15	0	0	0
20	0	0	0
25	0	2	46
30	0	2	46
35	0	83	83
40	0	47	47
45	0	24	17
50	0	0	0

Although no growth was shown at 10°C. in the first forty-eight hours, by the end of six days the majority of the spores had germinated and the hyphae had grown to about 290 microns in length, and at the end of three weeks the streaks were 5 mm. wide. At 15°C. this form showed no activity up to 72 hours, but

at 96 hours the spores had germinated and the hyphae had grown to an average of 290 microns in length. The growth of A. terreus was, in general, much different to that of A. flavus, and it would seem that its optimum growth temperature, 35°C., is a little higher.

Thermogenesis.

Aspergillus terreus appeared to be somewhat slower in actual growth than A. flavus under the conditions of the experiment, but its ability to produce heat was at least equal.

Table V. Thermogenesis and CO_2 production for Aspergillus terreus on alfalfa hay. Forty per cent moisture content.

	Flask 1		Flask 2		Flask 3		Flask 4			
Day	Temp. °C.	CO_2 mgs.	Temp. °C.	CO_2 mgs.	Temp. °C.	CO_2 mgs.	Temp. °C.	CO_2 mgs.	Check: °C.	Room °C.
0	27		27		27		27.0		26	24
1	27.5	1.4	28.5	1.4	28	1.4	27.5	1.3	24	25
2	29	1.7	31	1.7	30	1.8	30	1.7	25	25
3	35	1.8	35	2.1	35	1.8	35.3	1.9	21	22
4	39.2	2.0	41	2.3	40.2	2.0	40	2.0	25	25
5	40	2.5	40	2.3	40	2.5	40	2.6	23	24
6	37	2.6	38	2.5	36	2.6	38	2.1	23	24
7	33	2.0	34	2.0	32	2.0	34	2.0	22	25
8	30	1.6	31	1.0	30	1.6	30	1.5	23	25

Observations made daily at 9:00 a.m. The temperatures are given in degrees Centigrade and CO_2 production in milligrams per hour.

Table VI. Thermogenesis and CO_2 production for Aspergillus terreus on alfalfa hay. Forty per cent moisture content.

Day	Hour	Flask 3a		Flask 16a		Check	Room
		Temp. °C.	CO_2 mgs.	Temp. °C.	CO_2		
0	9:00 a.m.	23		23		23.3	23
1	9:00	24.4	.5	23.8	1	23	23
2	9:00	33.3	.6	32.9	1	23.8	23.8
3	9:00	34.1	1.5	33.3	2	24.4	24.8
4	9:00	33.2	1.5	33	2	22.7	23.3
5	9:00	33.5	2.0	33.2	2	23.3	23.8
6	9:00	32.9	2.1	32.9	1.5	23.0	24.0
7	9:00	31.5	2.1	31.	2	21	23.8
8	9:00	24	2	25	2	23	23

The maximum temperature reached was 41°C. on the fourth day. Norman (18) reported a similar maximum for A. terreus and showed that this organism was very active on cellulose and on hemicellulose, and able to use these substances as a source of carbon.

When the flasks were opened the organism was recovered and no invaders were found.

Aspergillus niger Van Tieghem

Though A. niger was not found on the hay from the heating mow it developed abundantly on every other sample. The culture used complied with the description of the group given by Thom and Church (23).

Growth index.

Difficulty was experienced in obtaining the growth index as some of the strains proved to be very slow growing, so slow at times, that at room temperature, growth could not be seen with the naked eye in a week. Other strains would germinate in a few hours and grow rapidly. This difference in growth habit is a varient which should be considered when attempting to determine the optimum growth temperature. At 30°C. the culture studied showed the most rapid growth for the individual hyphae, but at the end of the forty-eight hour period less than 70 per cent of the spores had germinated. While at 35°C. there was a somewhat slower growth of the hyphae, at the end of the twenty-four hour period only a very few ungerminated spores could be seen. Taking everything into consideration it would seem that 35°C. rather than 30°C., as appears on the table, would be nearer the optimum temperature.

Table VII. Growth index of A. niger as expressed in μ/hrs.

Temp. C.	1-8 hrs.	9-24 hrs.	25-48 hrs.
5	0	0	0
10	0	3	6
15	0	3	7
20	0	9	121
25	0	12	114
30	16	286	333
35	10	200	321
40	6	248	144
45	0	4	

After forty-eight hours the colonies continued to spread and spores to be formed. This continuation of activity was reflected in the heat curve which also continued to rise until the fifth day when a sharp decline occurred, after which, a level a few degrees above the check was maintained for several days.

Thermogenesis.

The maximum temperature observed under the conditions of the experiment was 39.4°C. Miehe (18), however, found that this organism would raise the temperature of grass hay to 48°C. Norman (19) obtained only a very slight rise on oat straw and because of this he thought that Miehe was not working with a pure culture. Other investigators have observed pronounced rises in temperature when this organism was placed on various substrate. For example, Gilman and Barron (7) observed 51.5°C. on wheat, 50°C. on barley and 52°C. on oats.

Under favorable conditions A. niger is actively thermogenic, though the amount of heat developed depends upon several factors, of which the type of substrate available is very important.

When the flasks were opened at the close of the experiment mycelium could be seen throughout the substrate. The organism was recovered and no invaders were found.

Table VIII. Heat development for Aspergillus niger given in °C.

Day	Hour	Waterbath 25 °C.		Laboratory table		
		Inoculated flask	Check	Flask 1	Flask 2	Check
0	10:00 a.m.	27	26	25	25	23
	5:00 p.m.	28				
1	8:00 a.m.	29	26	31.3	30.7	22.2
	1:00 p.m.	30				
	7:00	34.5				
2	8:00 a.m.	36	25	30.1	30.6	22.3
	1:00 p.m.	38				
	4:00	38.4				
	9:00	38.4				
3	8:00 a.m.	38.3	26	31.1	31.6	24.4
	12:00 m.	38.5				
	9:00 p.m.	38.8				
4	8:00 a.m.	38.9	26	32	32.5	23
	12:00 m.	38.6				
	4:00 p.m.	38.8				
5	8:00 a.m.	38.9	26	34.1	34.6	21
	1:00 p.m.	38.9				
	7:00	39.3				
	10:00	39.4				
6	8:00 a.m.	38	25	28	29	22
	5:00 p.m.	34				
7	8:00 a.m.	37	25	28	28	21
	5:00 p.m.	32				
8	8:00 a.m.	28	25	27	27	21

Table IX. Thermogenesis and CO_2 production for Aspergillus niger on alfalfa hay. Forty per cent moisture content.

Day	Hour	Flask 1		Flask 2		Check	Room
		Temp. °C.	CO_2 mgs.	Temp. °C.	CO_2 mgs.		
0	4:00 p.m.	25		25		26	24
1	9:00 a.m.	26	.4	26	.4	25	23
2	9:00	28	2.0	28	1.9	25	25
3	9:00	30.5	2.0	31	2.2	25	24
4	9:00	34	2.0	35	2.0	24	24
5	9:00	34	2.0	34	2.5	24	23
6	9:00	29	3.0	30	2.8	24	24
7	9:00	27	2.5	29	2.0	24	24.5
8	9:00	26	2.5	27	2.0	24	24

Aspergillus fumigatus Fresenius

The culture of A. fumigatus from which all the subcultures used in the investigation were made, was obtained from the heated hay in the experimental mow. The organism conformed to the description given in Thom and Church (23).

Although A. fumigatus was one of the forms which survived heating in the mow, it proved disappointing as far as heating ability was concerned when placed in pure culture on hay.

Growth index.

The table shows the optimum temperature for growth to be about 40°C., though good growth occurs from 25°C. to 45°C. and some growth at 50°C.

Table X. Growth index of A. fumigatus as expressed in μ/hrs.

Temp. °C.	1-8 hrs.	9-24 hrs.	25-48 hrs.
10	0	0	0
15	0	0	1
20	0	0	0
25	0	3	40
30	0	2	40
35	0	40	40
40		58	64
45	0	58	
50	Poor growth and no spores		

One can see from the above table that A. fumigatus not only tolerated but preferred higher temperatures than is usual for other fungi. At 40°C., while no germ tube was put out during the first eight hours, the spores had swollen to almost three times their original size.

Since no adequate method of controlling the moisture content of the media was devised, lack of moisture may have been the limiting factor at 45°C. and 50°C. after the 24-hour period.

At 20°C. and lower, the lag period was very pronounced, but growth took place. An interesting observation was made at 10°C. A culture had been examined frequently and no change took place in a week. The dishes were pushed back in the refrigerator and accidently left for three weeks. At the end of this period good growth had occurred and also abundant sporulation taken place.

Thermogenesis.

Several unsuccessful attempts were made to obtain heating

with A. fumigatus. At no time did the temperature of any inoculated flask rise more than 5°C. above the check. This has not been the experience of other workers and temperatures as high as 57°C. have been reported.

Table XI. Temperature and CO_2 production of Aspergillus fumigatus on alfalfa hay. Forty per cent moisture content.

Day	Flask 1		Flask 2		Check	Room
	Temp. °C.	CO_2 mgs.	Temp. °C.	CO_2 mgs.		
0	21.6		21.6		21	17.8
1	22.3	2.1	22.3	2.9	22	24
2	23.9	1.3	23.4	2.2	22	23
3	25.6	1.9	25.6	2.1	23	25
4	24.5	1.6	24.6	1.6	23	23
5	25	1.8	24.5	0.5	23	23.5
6	25.6	1.4	25	3.9	24	24
7	27.3	1.8	26.7	2.4	23	27
8	26	2.0	26	2.0	21	25

Readings taken at 9:00 a.m.

It is probable that in the experiments reported at this time, the temperatures at which A. fumigatus becomes active were never reached. There is also the possibility that the strain worked with was not an active one in producing heat. Then, again, it may have been that the substrate was the limiting factor.

It was observed that comparatively large amounts of CO_2 were produced and that the amounts varied widely between the inoculated flasks. The differences were especially notable when the temperatures of the flasks were compared.

When the flasks were unpacked, the mycelial growth was visible to the naked eye and the organism was recovered from platings. No invaders were found.

Aspergillus clavatus Desm.

Aspergillus clavatus appeared very frequently on platings, but as a rule its appearance was somewhat delayed. In cultural and morphological characteristics, it conformed to the description in Thom and Church (23).

Growth index.

The optimum growth temperature for A. clavatus appeared to be about 30°C., although good growth was made at all temperatures from 20°C. to 40°C. inclusive. At 15°C. a rapid growth took place during the 25-48 hour period, but at this time only about 50 per cent of germination had taken place.

Table XII. Growth index of A. clavatus as expressed in μ/hrs.

Temp. °C.	1-8 hrs.	9-24 hrs.	25-48 hrs.
10	0	0	3
15	0	5	78
20	0	2	39
25	3	6	50
30	15	24	80
35	0	40	40
40	0	4	12
45	0	3	
50	0	0	0

After 48 hours microscopic measurements were no longer possible. At 20°C. almost 100 per cent germination took place. This resulted in crowding which to some extent interfered with

the growth of the individual hyphae. The growth index as based on the increase in length of the individual hyphae, appeared greater at 15°C. than at 20°C., though there is no doubt that the aggregate growth for the number of spores seeded was much higher at 20°C. The growth response of A. clavatus was analogous to that of Penicillium oxalicum, though not quite so pronounced.

Thermogenesis.

Like A. fumigatus, A. clavatus produced a very small rise in temperature under the conditions of the experiment. At no time did the temperature rise more than 2°C. above the check. In the production of carbon dioxid, A. clavatus was very active.

Table XIII. Temperature and CO_2 production of Aspergillus clavatus on alfalfa hay. Forty per cent moisture content.

Day	Hour	Flask 1		Flask 2		Check	Room
		Temp. °C.	CO_2 mgs.	Temp. °C.	CO_2 mgs.		
0	10:00 a.m.	23		23		23.3	23
1	9:00	24.48	2.3	23.93	1.55	23.4	23.2
2	9:00	24.48	0.83	23.06	1.9	23.1	23
3	9:00	23.37	3.1	23.93	3.0	23.5	23
4	9:00	24.45	4.9	24.48	3.0	23.5	24
5	9:00	25.3	1.5	25.5	1.0	23	23
6	9:00	26.3	2.0	26.9	1.0	24	24
7	9:00	24.0	2.0	24.0	1.0	23	23

When the flasks were opened a good growth of mycelium was fairly well distributed through the hay, and sporulation had taken place near the top. By plating, the organism was recovered and no invaders were found.

Penicillium oxalicum Currie and Thom

Colonies of P. oxalicum occurred frequently on platings made from stored hay. The cultural and morphological characteristics complied with those given for P. oxalicum by Thom (22).

Growth index.

The form grew well over a wider temperature range than is usual for species of Penicillium. It was found to be rather difficult to obtain accurate measurements of the developing hyphae as they penetrated the substrate very early in their development and could not be followed. When growth for the first forty-eight hours only was considered, it appeared that 30°C. was the optimum temperature, but larger colonies developed at 25°C. in seventy-two hours. Twenty-five degrees Centigrade is probably nearer the optimum for growth.

Table XIV. Growth index of P. oxalicum as expressed in μ/hrs.

Temp. °C.	1-8 hrs.	9-24 hrs.	25-48 hrs.	49-72 hrs.
5	0	0	0	0
10	0	2	7	18
15	0	3	11	71
20	2	5	12	30
25	0	30	45	Colonies 10 mm.
30	0	24	42	" 8 mm.
35	1	70	38	" 5 mm.
40	0	0	0	0
45	0	0	0	0

If table XIV is examined, a sharp decrease in the growth index between the 15°C. and the 20°C. interval, during the 49-72 hour period, may be seen. This condition was contrary to expectation and appeared to be entirely out of line. A closer examination of the condition of the experimental plates revealed a situation which may explain the wide deviation. At 15°C. only about 50 per cent of the spores germinated. After germination 15°C. was a favorable growth temperature and the hyphae were able to grow rapidly for some time before crowding became an inhibiting factor. On the other hand, we had for P. oxalicum at at 20°C. a still more favorable growth temperature, and we had also approximately 100 per cent germination. With greater germination and more rapid growth, a crowded condition occurred which in turn inhibited the growth of individual hyphae. The accelerating effects of increased temperature were inadequate to overcome the inhibiting effects of crowding, and a decrease in the growth index resulted when the increase of the individual hyphae, only, were considered. If, however, accurate measurement could have been made, in all probability it would have been found that more mycelium had developed at 20°C., per spore seeded than at 15°C.

Table XV. Temperature readings and CO_2 production for *Penicillium oxalicum* when grown on hay with 40 per cent moisture content.

Day	Hour	Waterbath 25°C. Temp. °C.	Waterbath 25°C. Check °C.	Flask 1 Temp. °C.	Flask 1 CO_2 mgs. per hr.	Flask 2 Temp. °C.	Flask 2 CO_2 mgs. per hr.	Check	Room
0	3:00 p.m.	25.3	26						
	4:00 p.m.			24		24		24	25
1	8:00 a.m.	29	27						
	9:00			28	0.3	27	0.4	24	23
	10:00	29.9							
	1:00 p.m.	30.2							
	5:00	30.4							
	10:00	30.8							
2	9:00 a.m.	31.2	26	30	0.6	29	0.6	24	24
	1:00 p.m.	31.4							
	5:00	31.4							
3	8:00 a.m.	32.3	26						
	9:00			31	1.0	31.5	1.2	24	22
	12:00 m.	32.5							
	5:00 p.m.	33.2							
4	8:00 a.m.	33.8	26						
	9:00			33	1.0	34	1.0	24	23
	1:00 p.m.	33.9							
	5:00	33.9							
5	8:00 a.m.	34.2	26						
	9:00			32	1.0	33	1.0	24	23
	4:00 p.m.	34.5							
	8:00	34.5							
6	8:00 a.m.	32.6	26						
	9:00			30	1.2	32	1.3	23	22
	1:00 p.m.	32.0							
	7:00	32.0							
7	9:00 a.m.			30	1.0	30	1.1	23	23
8	9:00 a.m.			29	1.0	28	1.0	23	23

Thermogenesis.

From the preceding table it will be seen that this species of Penicillium was fairly active in heat production although it did not produce relatively large amounts of CO_2. The maximum temperature, 34°C., or 10°C. above the check, was reached on the fourth day. The production of CO_2 continued after the decline.

If the heat curve, shown in Plate IV fig. 2, is examined it will be noted that there was a rather rapid rise which continued until the maximum was reached. This period of heat formation corresponded with the rapid growth period for the organism on artificial media.

Penicillium humicola Oudemans

The organism carried under the name Penicillium humicola complied with the description given by Gilman and Abbott (8). It was frequently encountered on plates made from stored hay.

Growth index.

Penicillium humicola grew well at much lower temperatures than P. oxalicum. By following the growth index method the optimum growth temperature was found to be about 20°C., though the growth was almost as good at 15°C. Some growth occurred as high as 35°C., but it was slow and only about 25 per cent of the

spores germinated. At 40°C. no germination whatever took place.

Microscopic measurements were impossible at the 20°C. and 25°C. intervals after the 48 hour period, but rapid growth continued and many spores were produced.

Table XVI. Growth index for P. humicola as expressed in μ/hrs.

Temp. °C.	1-8 hrs.	9-24 hrs.	25-48 hrs.	49-72 hrs.
10	0	0	2	11
15	0	4	36	100
20	0	27	42	
25	2	15	25	
30	0	3	5	
35	0	2	2	
40	0	0	0	

Thermogenesis.

Though several experiments were made on thermogenesis with P. humicola, the results on each occasion were very similar and varied only within narrow limits. One set of observations was made on a culture before it was finally identified as a duplicate isolation of P. humicola. The results in this case were also very similar. Only one representative table is given.

At no time did P. humicola raise the temperature of its substrate more than 2°C. The evolution of CO_2 was generally rapid though it fluctuated greatly, and as far as heat production was concerned, it was meaningless.

Table XVII. Temperature and CO_2 production of Penicillium humicola on alfalfa hay. Forty per cent moisture content.

Day	Hour	Flask 17		Flask 8		Check	Room
		Temp. °C.	CO_2 mgs.	Temp. °C.	CO_2 mgs.		
0	10:00 a.m.	24		24		24	24
1	9:00	27	1.0	24	.56	24	23
2	9:00	26	0.9	24	1.33	23	22
3	9:00	26	1.3	23	1.25	24	25
4	9:00	23	1.4	20	1.32	21	21
5	9:00	26	0.46	26	0.94	24	26
6	9:00	23	1.39	23	1.24	23	23
7	9:00	25	1.32	25	1.56	23	25
8	9:00	24	1.0	24	1.0	24	23

Spicaria divaricata (Thom) Gilman and Abbott

Low, buff colored colonies of S. divaricata appeared rather frequently on plates, usually after incubation for one week. It conformed to the description given in Gilman and Abbott (7).

Growth index.

Spicaria divaricata grew over a wide temperature range and after one week little difference could be seen in the size of the colonies grown at the temperature intervals from 20°C. to 40°C. inclusive. The optimum appeared to be about 30°C., but even at this temperature the colonies attained a diameter of only 10 mm. in eight days.

Table XVIII. Growth index of S. divaricata as expressed in μ/hrs.

Temp. °C.	1-8 hrs.	9-24 hrs.	25-48 hrs.	49-72 hrs.
10	0	7	4	3
15	0	7	4	9
20	0	18	35	250
25	0	15	30	
30	0	17	35	300
35	0	10	30	
40	0	10	76	200

Thermogenesis.

Table XIX. Temperature and CO_2 production of Spicaria divaricata on alfalfa hay. Forty per cent moisture content.

Day	Hour	Flask 1		Flask 2		Check	Room
		Temp. °C.	CO_2 mgs.	Temp. °C.	CO_2 mgs.		
0	10:00 a.m.	29		28.5		29	30
1	9:00	29	0.3	28	0.3	29	28
2	9:00	28	0.55	28	0.45	27	28
3	9:00	27	0.5	28		27	26
4	9:00	26		27	0.3	26	27
5	9:00	27	0.3	27		27	27
6	9:00	27	0.1	27	0.1	27	28
7	9:00	27	0.1	26	0.1	26	27
8	9:00	28	0.1	27	0.1	27	29

The amount of heat produced by S. divaricata was negligible and the amount of CO_2 was also very small, so small that the accuracy of the measurement is doubtful. At the close of the experiment there was no visible evidence of fungal growth in the mass, though the organism was recovered by plating.

In this case we have an organism which grew so slowly that if heat was generated at all it was never in excess of the amount lost from the flask by radiation.

By reference to the graph on Plate VI fig. 1 showing the amounts of heating and CO_2 evolution in Spicaria divaricata, it is interesting to note that the curve for CO_2 evolution resembles very closely that for heat production.

Mucor abundans Povah

The original culture of M. abundans was obtained by transferring to an agar slant, a piece of sterile mycelium which was abundant on the hay samples. On artificial media it fruited and subcultures were made by touching the sporangiophores with a needle and transferring the spores to agar slants. In cultural and morphological characteristics, the culture conformed to the description in Gilman and Abbott (7).

Growth index.

Evaluation of the growth index for M. abundans was difficult because of its straggly growth habits. At first the growth was close to the substrate, then after about one week, aerial hyphae were sent up and sporophores developed. This form grew well at all temperatures from 15°C. to 40°C., but at 45°C. no germination took place. The greater number of spores germinated at 30°C.

Table XX.
Growth index of M. abundans as expressed in μ/ hrs.

Temp. °C.	1-8 hrs.	9-24 hrs.	25-48 hrs.
5	0	0	0
10	0	1	13
15	0	0	6
20		22	114
25	6	50	130
30	0	97	127
35	0	80	110
40	10	150	100

Thermogenesis.

Table XXI. Temperature and CO_2 production of Mucor abundans on alfalfa hay. Forty per cent moisture content.

Day	Hour	Flask 1		Flask 2		Check	Room
		Temp. °C.	CO_2 mgs.	Temp. °C.	CO_2 mgs.		
0	12:00 m.	22.4		22.5		22	20
1	9:00 a.m.	24.1	1.4	24.0	1.0	22	21
2	9:00	26.3	1.6	25.5	1.4	21	23
3	9:00	27.4	2.0	27.0	2.0	22	23
4	9:00	28.5	2.0	28.0	2.0	22	22
5	9:00	24.0	2.4	25.0	2.0	20	21.5
6	9:00	24.0	2.0	24.0	2.0	19	19
7	9:00	24.0	1.0	23.0	1.0	19	19
8	9:00	26.0	1.5	23.0	1.0	20	22

Mucor abundans developed some temperature, at one time reaching 28.5°C., which was 6.5°C. above the check. It was also active in the production of CO_2 and produced more in comparison than some other forms which produced greater amounts of heat. Miehe (18) reports one species of Mucor which he found capable of raising the temperature of its substrate to over 50°C. Another

species of Mucor, which was lost after some preliminary experiments, and before it was identified, produced 34.5°C. or 10°C. above the check, on the fifth day.

When the flasks were unpacked the hay was almost covered with the white mycelium. Examination and platings showed no invaders present.

Note: To inoculate the hay with a Mucor, some of the mycelium was placed in the substrate by means of an inoculating needle, in addition to the spore suspension.

Rhizopus tritici Saito

Rhizopus tritici was the only species other than those of the genus Aspergillus that survived the heating in the experimental mow. A description of the organism complied with that given by Lendner (16).

Growth index.

When grown on artificial media R. tritici spread out over the substrate with much branching, and yet produced only a very small amount of mycelium. Growth took place at all temperatures from 20°C. to 45°C., with the optimum at about 35°C. Table XXII will show the trends of growth response to temperature.

Table XXII. Growth index of R. tritici as expressed in μ/hrs.

Temp. °C.	1-8 hrs.	9-24 hrs.	25-48 hrs.
10	0	2	16
15	0	12	70
20	0	0	187
25	5	200	350
30	0	60 mm.	100 mm.
35	8	60 mm.	100 mm.
40	12	85	100 mm.
45	0	80	

Thermogenesis.

For the experiments to find the thermogenic ability of R. tritici a series of five flasks were prepared. Three of these were inoculated with a spore suspension and two prepared as checks. One inoculated flask with its check was placed in the constant temperature bath and the temperature of each read with thermometers. Two other inoculated flasks with a check were held at room temperature and their temperature changes read by means of a thermocouple. The temperature readings and also the amounts of CO_2 produced are recorded in Table XXIII.

The maximum temperature, 42.9°C., was attained on the second day in the flask held in the water bath. Flasks 1 and 2 reached 42°C. and 41°C. respectively on the third day. It is reasonable to believe that the peaks in flasks 1 and 2 may have been missed. After the third day the temperature of each of the inoculated flasks fell rapidly to a few degrees above the check where it remained for several days.

Table XXIII. Temperature readings and CO_2 production for *Rhizopus tritici* when grown on hay with 40 per cent moisture content.

Day	Hour	Waterbath 25°C. Flask 3 Temp. °C.	Waterbath 25°C. Flask 3 Check °C.	Laboratory tables Flask 1 Temp. °C.	Laboratory tables Flask 1 CO_2 mgs.	Laboratory tables Flask 2 Temp. °C.	Laboratory tables Flask 2 CO_2 mgs.	Check Temp. °C.	Room Temp. °C.
0	10:00 a.m.	27	27	25		26		25	26
1	8:00 a.m.	34.5	26	30	1.5	31	1.7	25	24
	12:00 m.	35.1							
	8:00 p.m.	35.9							
2	8:00 a.m.	40	26	40	2	39	2.1	25	25
	11:00	42.6							
	5:00 p.m.	42.9							
	10:00	42.6							
3	8:00 a.m.	41	26	42	2.2	41	2.1	25	24
	11:00	38							
	2:00 p.m.	35							
4	8:00 a.m.	32	25	33	2.3	35	2.2	25	25
5	8:00 a.m.	30	25	30	1.6	31	1.5	25	25
6	8:00 a.m.	30	25	30	1.0	30	1.1	25	24
7	8:00 a.m.	29	25	29	1.1	28	1.2	25	24
8	8:00 a.m.			27	1.0	27	1.0	25	24

Rhizopus tritici, under favorable conditions, will germinate almost immediately and make a rapid, spreading growth, covering the entire substrate. When the flasks were unpacked at the close of the experiment, the mycelium could be seen mixed throughout the contents. The study on temperature response shows a very rapid period of growth during the 25-48 hour period. If Table XXII and Table XXIII are compared it will be seen that the rapid growth period corresponded to the period of rapid heat production.

Rhizopus nigricans Ehrenberg

Rhizopus nigricans is a very common form in nature and it was encountered very frequently on alfalfa hay. When its growth index was obtained, it was found to have a narrower growth temperature range than the majority of forms studied. In cultural characteristics it conformed to the description in Gilman and Abbott (7).

Growth Index.

Rhizopus nigricans germinated and grew, though very slowly, at 5°C. From 10°C. on it showed distinctly increased growth for each increase of temperature up to 25°C., but at 30°C. no germination whatever occurred.

Table XXIV. Growth index of R. nigricans as expressed in μ/hrs.

Temp. °C.:	1-8 hrs. :	9-24 hrs. :	25-48 hrs. :	49-72 hrs.
10	0	5	6	36
15	0	14	56	479
20	9	38	dish covered	
25	7	60	" "	
30	0	0	0	0

Thermogenesis.

To study temperature and CO_2 production, four flasks were prepared in the usual manner, three of these were inoculated and the fourth left for a check. All temperature readings were made by means of the thermocouple.

Table XXV. Temperature readings and CO_2 production for Rhizopus nigricans on alfalfa hay. Forty per cent moisture content.

Day:	Flask 1		Flask 2		Flask 3		Check	Room
	Temp.: °C.	CO_2: mgs.	Temp.: °C.	CO_2: mgs.	Temp.: °C.	CO_2: mgs.		
0	23		23		24		23	22
1	22	1.8	23	1.8	26	2	23	22
2	23	2	23	1.8	37	1.5	23	23
3	25	1.8	24	2.2	36	1.9	23	22
4	28	1.8	28	3	30	2	23	24
5	34	2	33	2.2	25	2.3	23	24
6	37	2	36	2.2	25	2	23	24
7	34	2.6	35	2.4	25	1	23	25
8	25	2	26	2	25	1	24	26

Experiment was started at 12:00 m. and temperatures read at 9:00 a.m. daily.

In the above table it will be noted that flask three of the experiment followed an entirely different course in the production

of heat than did flasks one and two. As far as is known, the experimental conditions for each flask were the same. The three large slants on which the inoculum was grown were seeded from the same agar slant. Each of the flasks was found to be uncontaminated and the type of growth on the substrate about the same.

The maxima of flasks one and two were 37°C. and 36°C., respectively, both on the sixth day. But for flask three, the maximum of 37°C. was reached and held throughout the second day. After reaching the maximum the temperature of flask three dropped rapidly to one or two degrees above the check, where it remained. In this case the evolution of carbon dioxid continued after the production of temperature had ceased.

It was especially noticeable in flask three that the rapid increase in temperature corresponded to the period of rapid growth on artificial media. In flasks one and two, once the temperature began to rise at the end of the lag period, the course and duration of the heat curves were very similar to that of flask one. In the graph, Plate III fig. 1, illustrating the curve for heat production and CO_2 evolution, the average of flasks one and two was used. If a graph of the curve for flask three were prepared it would show a similar curve to that of flasks one and two except that the rise would begin earlier.

Cunninghamella elegans Lendner

Cunninghamella elegans was an example of a form which, though unable to survive high temperatures, could itself release a great amount of heat from its substrate. It grew abundantly on the wetted hay and also on the plated washings. It complied to the description for C. elegans given by Lendner (16).

Growth index.

Cunninghamella elegans was probably one of the most interesting forms studied. It grew well over a wide temperature range and produced a large amount of mycelium. In forty-eight hours the petri dishes were entirely filled at each temperature interval from 20°C. to 40°C. At 45°C. a little growth took place in twenty-four hours. After this period growth stopped due to drying of the media. In table XXVI will be seen, with the exception of a slight drop at 35°C., an increase in growth for each increase of temperature. The growth index for the 25-48 hour period was, of course, difficult to evaluate by the method used.

Table XXVI. Growth index for C. elegans as expressed in μ/hrs.

Temp. °C.	1-8 hrs.	9-24 hrs.	25-48 hrs.
10	0	0	30
15	0	51	103
20	0	85	173
25	8	200	Dishes filled with
30	5	416	mycelium and spores
35	7	400	formed.
40	0	555	

Thermogenesis.

Table XXVII. Temperature readings for C. elegans taken with the thermometer.

Day	Flask 7 - Temp. °C.	Flask 8 - Temp. °C.	Check
0	25	25	25
1	28	28	26
2	40.1	40	26
3	31.4	31	23
4	30	30.8	22
5	29.6	30.3	22
6	30.8	30	23
7	29.3	29	23
8	23	25	23

Flasks packed at 10:00 a.m. Temperatures read at 9:00 a.m.

Table XXVIII. Temperature readings and CO_2 production for Cunninghamella elegans when grown on hay with 40 per cent moisture content.

Day	Hour	Flask 1		Flask 2		Check	Room
		Temp. °C.	CO_2 mgs.	Temp. °C.	CO_2 mgs.		
0	4:00 p.m.	26		26		26	28
1	9:00 a.m.	27	2	27	2.1	26	26
2	9:00	30	2	29	2	26	26
3	9:00	36	2.5	37	2.6	26	25
4	9:00	30	3.3	31	3	26	26
5	9:00	30	2	29	3	26	22
6	9:00	30	3	29	3	24	23
7	9:00	29	3	29	2	24	24
8	9:00	25	2.5	25	2	24	24

The data obtained from the experiments on thermogenesis and recorded in the two tables above were procured on different occasions several weeks apart. Table XXVII is the result of an experiment carried on in which thermometers were used. The room

temperature during this experiment was not kept, but this was not a very serious omission. The check showed a downward trend which was, no doubt, a response to environmental conditions. In the inoculated flasks a maximum temperature of 40.1°C. or 14°C. above the check was shown on the second day. A rapid rise in temperature from 25°C. to 40°C. occurred in forty-eight hours; this rise corresponded closely with the period of rapid growth and accumulation of mycelium on artificial media, as may be seen by comparing table XXVI with table XXVII.

The thermocouple readings showed a little lower temperature, but if we compare table XXVII with table XXVIII it is easy to believe that the maxima were missed and that they occurred between the second and the third days. Carbon dioxid evolution was continued in relatively large quantities after the heat maximum had been passed.

When the flasks were unpacked, the mycelium could be plainly seen with the naked eye; it had spread intricately and had involved the entire contents. The organism was reisolated from each of the inoculated flasks by plating.

Hormodendron nigrescens Paine

Hormodendron nigrescens was not observed at all on the wetted hay but appeared persistently on platings. The description of the organism complied with that given in Gilman and Abbott (7).

Growth index.

Hormodendron nigrescens grew very slowly with hard, compact, round colonies at the room temperatures prevailing at the time of isolation. At lower temperatures it would grow much more rapidly and spread out over the medium. No germination was obtained above 30°C. Due to the fact that this wasavery slow growing form, it was possible to extend measurements of individual hyphae over the 49-72 hour period.

Table XXIX. Growth index of H. nigrescens as expressed in μ/hrs.

Temp. °C.	1-8 hrs.	9-24 hrs.	25-48 hrs.	49-72 hrs.
5	0	0	10	30
10	0	10	20	68
15	0	20	26	146
20	10	10	11	114
25	0	0	0	10
30	0	0	0.5	0.25
35	0	0	0	0
40	0	0	0	0

As may be seen from the above table the optimum growth took place at 15°C.

Thermogenesis.

At the time the following data were obtained, the weather was a little cooler than it had been during the majority of the observations on other forms. This greatly influenced the initial temperature of the flasks. At the time of packing the temperature

of the flasks stood between 21°C. and 22°C., which is near the temperature most favorable for growth for the form under observation.

Table XXX. Temperature readings and CO_2 production for Hormodendron nigrescens when grown on hay with 40 per cent moisture content.

Day	Hour	Flask 1		Flask 2		Check	Room
		Temp. °C.	CO_2 mgs.	Temp. °C.	CO_2 mgs.		
0	12:00 m.	21.1		22.2		21	25
1	8:00 a.m.	24.5	.68	24.2	.76	20.5	24
2	8:00	25.5	2.93	24.8	3.42	20.5	24
3	8:00	25.5	2.26	24.8	1.67	20.5	24
4	8:00	23.8	1.59	21.8	1.24	18.3	24
5	8:00	23.8	0	21.9	1.9	18.9	23
6	8:00	25.5	.47	25.3	2.17	21.1	23.5
7	8:00	22.5	1.95	24.5	1.36	22.7	23
8	8:00	26.7	1.0	25.3	.95	22.7	23

The temperature of the flasks rose to about four degrees above the check and remained at this point during the eight days of observation. It will be noted that the production of CO_2 fluctuated over a rather wide range, at one time becoming so low as to make accurate measurement doubtful, with the methods used.

At the end of the experiment the organism was recovered by plating although no mycelium was perceptable in the mass.

In Hormodendron nigrescens we have another case in which an organism that preferred lower temperatures for its best growth produced little heat. The evolution of CO_2 indicated that a great deal of activity was taking place in the flasks, which, however, did not result in the accumulation of heat.

Alternaria humicola Oudemans

Although A. humicola was never observed growing on the hay samples, colonies were very frequently found on platings made from them. The form isolated complied with the description given in Gilman and Abbott (8).

Growth index.

The culture isolated was able to adjust itself to a wide temperature range and spores germinated and grew at each temperature interval from 0°C. to 40°C.

Table XXXI. Growth index for A. humicola as expressed in μ/hrs.

Temp. °C.	1-8 hrs.	9-24 hrs.	25-48 hrs.
0	3	3	2
5	2	14	23
10	9	82	23
15	10	80	100
20	10	72	82
25	60	200	100
30	53	287	63
35	4	30	70
40	2	5	30
45	0	0	0

Measurements of the growing hyphae were extremely difficult in this form owing to various types of germination and growth. Some spores would put out several germ tubes and the hyphae would branch very early. In others, the germ tubes were limited

to the basal and the apical cells. There was no uniformity in the type of branching under any condition, and all types of germination and growth were found at each temperature at which growth took place.

The optimum growth temperature was found to be between 25°C. and 30°C. at which temperatures the spores germinated immediately when placed on artificial media, and a new crop was produced in twenty-four hours. At 15°C. and 20°C. there was also almost 100 per cent germination with several germ tubes from each spore, but about forty-eight hours were required to complete the cycle.

Thermogenesis.

Under the conditions of the experiment Alternaria humicola never raised the temperature of the substrate over 3°C. above that of the check, but it produced comparatively large amounts of CO_2. Observations were repeated several times with very similar results. The following table is representative.

Temperature production was never over two or three degrees above the check, but the amount of CO_2 produced was greater than that of Aspergillus flavus, one of the most actively thermogenic organisms. The production of CO_2 also fluctuated over a rather wide range, even though the amounts of temperature remained rather even.

Table XXXII. Temperature readings and CO_2 production for Alternaria humicola when grown on hay with 40 per cent moisture content.

Day	Hour	Flask 3		Flask 4		Check	Room
		Temp. °C.	CO_2 mgs.	Temp. °C.	CO_2 mgs.		
0	4:00 p.m.	23		23		23	24
1	8:30 a.m.	23	0.6	23	0.6	23	22.5
2	8:30	25.5	1.8	26	2.1	23	24
3	8:00	25.5	3.8	26	4.1	24	25
4	8:00	25	1.9	25.5	2	24	25
5	8:00	25.5	4.4	25.5	4.9	24	25
6	9:00	25	3.5	25	2.7	23	23
7	10:00	25	4.6	25	4.3	24	25
8	5:00 p.m.	27	5.2	27	1.1	25	27

The data indicate that under the conditions extant at the time, the measure of CO_2 production gave no indication of the amount of heating.

At the close of the experiment the organism was recovered in pure culture. No invaders were found.

DISCUSSION

Growth Index

As explained under materials and methods, the growth index for each organism was obtained by measuring its actual growth increment in microns for a given time and dividing the number of microns obtained by the number of hours required to make the increase. When this method is used there are several variable factors which must be taken into consideration. Often a species is composed of several strains which differ from each other in the time required for germination, and the rate of growth. During the present studies we found one strain of Aspergillus niger that required almost a week before any germination or growth took place; another strain, which conformed to the same general description as the above, germinated and grew immediately when placed on suitable media. Some species, especially those of the Mucorales, produced a rapid but sparse growth which spread all over the culture. When the hyphae were measured the growth indices appeared high. In other forms the hyphae would penetrate the substrate very early and be lost. There were also many types of branching which made microscopic measurement hard to evaluate.

The microscopic growth index method will find its greatest usefulness when the response of a single organism to changed conditions of growth is required. In the present study a fairly accurate picture of the growth temperature range of each individual was obtained, then by comparison of the growth indices for various temperature intervals, the optimum growth temperature was established. Further, by comparing the growth indices for different periods of time, a time period when the most rapid growth occurred could be defined. As the investigation proceeded it was found that the period of active growth on artificial media approximated the period of most rapid accumulation of heat for several of the organisms studied.

In general, each form studied increased its growth activity with increased temperature until the optimum was reached, after which a sharp decreased occurred. However, there were some apparent exceptions, for example, Penicillium oxalicum. In the study of this form it was found that at 10°C. the growth index was 18, at 15°C. it was 71 and at 20°C., a very favorable growth temperature, the growth index was only 30. A closer examination of the experimental plates showed that at 15°C. only 50 per cent of the spores had germinated, but at 20°C. germination was almost 100 per cent. Increased germination resulted in crowding and inhibition of the growth of the individual hyphae. Microscopic measurement of the individual hyphae gave a greater growth index at 15°C. than at 20°C., but there was no doubt that if the amount

of mycelium per spore seeded could have been accurately determined, it would have been much greater at 20°C. It was purely a case where the acceleration of increased temperature was neutralized by the inhibiting effects of crowding. Several forms, especially those which tolerated or preferred the higher temperatures, were able to grow over a wide temperature range. A good example was Aspergillus fumigatus, which had its optimum above 40°C., was able to make growth at 50°C., and, if allowed sufficient time, was also able to grow well at 10°C. The growth activity of Cunninghamella elegans was very interesting. At all the temperature intervals from 10°C. to 40°C. rapid growth occurred. From 25°C. to 40°C. the petri dishes were completely filled with mycelium in forty-eight hours. Another form, Spicaria divaricata, grew over a wide range of temperature, though the amount of mycelium produced at any time was very small. Two other forms, Hormodendron nigrescens and Penicillium humicola, preferred temperatures below 20°C. The following table shows the optimum temperature for each form studied as determined by the growth index method.

Table XXXIII. Growth optima of organisms studied.

15°C. -20°C.	25°C.	30°C. - 35°C.	40°C.
Hormodendron nigrescens	Rhizopus nigricans	Aspergillus flavus	Aspergillus fumigatus
Penicillium humicola	Alternaria humicola	A. terreus	
	Penicillium oxalicum	A. clavatus	
		A. niger	
		Rhizopus tritici	
		Mucor abundans	
		Spicaria divaricata	
		Cunninghamella elegans	

Thermogenesis

In all the experiments in thermogenesis alfalfa hay was used as the substrate. The severe treatment of the hay in sterilization may be open to some criticism because of chemical changes brought about during the process. All hay used in the experiment was treated in exactly the same manner; therefore, whatever benefits or handicaps accrued were the same for each organism studied and would not seriously affect the results. It was obvious that no living tissue of the hay itself survived.

The data which have been obtained showed that certain fungi were able to raise the temperature of alfalfa hay after all factors, other than those involved in the vital processes of the fungi themselves, had been eliminated. All the forms studied were not equal in their power to release heat; in fact, the thermogenic ability of some was almost negligible under the conditions of the experiment. These non-thermogenic species were usually either very slow in growing or they preferred lower temperatures for their maximum activities. It was easy to believe that such forms produced heat but that it was liberated no faster than it was dissipated. Plate VI fig. 1 shows graphically that very little heat was produced by Spicaria divaricata. While this form grew over a wide temperature range, it only produced a small amount of mycelium. Another form, Hormodendron nigrescens, as shown in Plate IV fig. 1, had for its optimum growth temperature 15°C. The temperature of the flask inoculated with H. nigrescens was at no time more than a few degrees above the check. It was possible that rising temperatures inhibited growth, which inhibition was reflected in the release of heat.

Aspergillus flavus was the most active heat producer on hay. When placed on any suitable medium it germinated quickly and grew very rapidly. This characteristic was reflected in the temperature produced. As may be seen in Plate II fig. 3, the temperature of a flask inoculated with A. flavus started to rise in a very short time, and continued until the maximum of 44°C. was reached

on the third to fifth day. After the maximum was reached there was a rapid decline to a few degrees above the check. The new level was held for several days if the flasks were not interfered with. Two other species of Aspergillus found to be definitely thermogenic were A. terreus and A. niger. Both these forms raised the temperature of their substrate to about 40°C. or 14°C. above the check. In the present experiment, A. fumigatus released very little heat. This was disappointing in view of the findings of several other investigators, namely, Miehe (18), James, Rettger and Thom (15), Norman (19), and Gilman and Barron (7). Aspergillus fumigatus was found by these workers to be very active on several types of substrate. For example, the data of Gilman and Barron show a maximum of 53.2°C. when grown on oats and of 31.2°C. on wheat. These data indicate that the type of substrate effects the amount of heat released by fungi. Hay, perhaps, was not a good substrate for A. fumigatus, or it may have been that the initial temperature was too low for the maximum activity of the fungus.

It was very interesting to note that two species of Rhizopus and one species of Mucor were thermogenic. Rhizopus tritici and R. niger raised the temperature of their substrates to 42.9°C. and 37°C. respectively. Mucor abundans reached only 28.5°C., but this point was 6.5°C. above the check. Miehe (18) reported 38°C. for R. nigricans and over 50°C. for Mucor corymbifer.

Another of the Phycomycetes, Cunninghamella elegans, was

found to be powerfully thermogenic. The series of experiments from which the data graphically recorded in Plate V fig. 3 was obtained, showed a maximum temperature of 37°C. on the third day, but in another observation over 40°C. was attained on the second day. This form grew rapidly over a wide range of temperature and in addition it produced a large amount of mycelium.

Of the species of Penicillium studied P. oxalicum was thermogenic while P. humicola was not. Studies on growth showed that P. humicola preferred a lower temperature for growth than did P. oxalicum. This difference in growth habit was reflected in the rapidity in which temperature was released.

The amount of temperature released by fungi should be studied on a quantitative basis by a series of experiments conducted with a calorimeter. The relative efficiency of each organism could, no doubt, be established.

A summarized list of the species studied for heat production with the maximum temperature attained, the difference between the maximum of the inoculated flask and the check, and the day on which the maximum was reached, for each form is given in Table XXXIV.

Table XXXIV. Maximum temperatures in degrees Centigrade developed by organisms grown on sterile alfalfa hay. Forty per cent moisture content.

Organisms	Maximum Temp.	Temp. of check	Maximum Temp. above check	Day
Aspergillus flavus Link	44.4	25	19.4	3
A. terreus Thom	41	27	14	4
A. niger Van Tieghem	39.4	26	13.4	6
A. fumigatus Fres.	27	24	3	7
A. clavatus Desm.	26.6	24	2.6	6
Penicillium oxalicum Currie and Thom	34	24	10	4
P. humicola Oud.	26	24	2	5
Spicaria divaricata (Thom) Gilman and Abbott	27	26	1	4
Mucor abundans Povah	28.5	22	6.5	5
Rhizopus tritici Saito	42.9	26	16.9	3
R. nigricans Ehrenberg	37	23	14	6
Cunninghamella elegans Lendner	40	26	14	2
Hormodendron nigrescens Paine	25.5	20.5	5	4
Alternaria humicola Oud.	27	24	3	5

Thermogenesis and CO_2 production.

The data showed in some cases a rather close similarity in the progress of the curve for CO_2 production to that of heating. In other cases the CO_2 curve was indicative of much greater activity than was shown by the temperature recorded. In no case could comparison of activity be made between different organisms, unit of CO_2 for unit of heat. This condition may be illustrated by comparing the tables for Aspergillus flavus (Table III) with those of Alternaria humicola (Table XXXII). Here it is found that Alternaria humicola, though it only produced enough heat to

raise the substrate 3°C. above the check, produced a much greater amount of CO_2 than was produced by Aspergillus flavus, which raised the temperature of its substrate almost 20°C. above the check. In Plate IV fig. 1, the curve for CO_2 production for Hormodendron nigrescens shows a very rapid rise to the second day followed by a rapid decline to the fifth day. Evidence of much more vital activity is shown by the amount of CO_2 produced than by the amount of heat measured. Norman (19) obtained a closer relationship between CO_2 production and heating, both with a mixed micro-flora and with a pure culture of Trichoderma sp. The factors influencing the relation between CO_2 and thermogenesis in the investigations here reported were not sufficiently controlled to allow of explanation of the discrepancy found.

When a substrate like hay was used, it was almost impossible to keep conditions for respiration constant, especially in closed vessels. Even with constant aeration, one had no guarantee against local matting of the mycelium or caking of the substrate. If one were able to section the mass he would find all gradations from aerobicand anaerobic conditions. Under anaerobic conditions degradation of carbohydrates would occur with the release of much smaller amounts of heat than under aerobic conditions.

Thermogenesis and growth.

Those forms of fungi found to be actively thermogenic, were, in general, capable of active growth and produced large amounts of mycelium. When the growth indices were compared with the

temperature production it was found that the period of rapid accumulation of temperature corresponded very closely with the period of rapid germination and growth on artificial media. This was well illustrated in the case of Cunnihghamella elegans, in which the growth index for the 1 to 8 hour period was 5 and for the 9 to 24 hour period rose to 416 and continued too rapidly for measurement. Table XXVII shows a very rapid rise in temperature from 25°C. to 40°C. in forty-eight hours. The data indicate that thermogenesis is subordinate to the vital processes involved in growth, and heat results when more energy is released by the organism than is required for its growth.

SUMMARY

1. A number of common hay-inhabiting fungi were isolated and studied.

2. The growth response of each form to temperature was studied on artificial media by measuring the growth increment of the hyphae and dividing by the number of hours required for the increase. This gave the growth index.

3. The growth optima obtained were: Hormodendron nigrescens and Penicillium humicola, 15°C. to 20°C.; Rhizopus nigricans, Penicillium oxalicum and Alternaria humicola, 25°C.; Aspergillus flavus, A. terreus, A. clavatus, A. niger, Rhizopus tritici, Mucor abundans, Spicaria divaricata and Cunninghamella elegans, 30°C. - 35°C.; Aspergillus fumigatus, 40°C.

4. Each organism was inoculated in pure culture on sterile alfalfa hay in thermos flasks, the substrate brought to 40 per cent moisture content, and thermogenesis and CO_2 evolution measured.

5. Each form was able to raise the temperature of its substrate to some degree. Aspergillus flavus, A. terreus, A. niger, Penicillium oxalicum, Rhizopus tritici, R. nigricans, and Cunninghamella elegans proved to be decidedly thermogenic. Mucor abun-

dans and Hormodendron nigrescens were also thermogenic, but to a lesser degree.

6. Under the conditions of the experiments, Aspergillus fumigatus, A. clavatus, Penicillium humicola, Spicaria divaricata and Alternaria humicola developed little or no heat.

7. Though the curve for CO_2 evolution in many cases simulated that for heating, CO_2 production could not be used as a measure of thermogenesis.

8. Periods of rapid accumulation of heat in the inoculated flasks corresponded to periods of active germination and growth on artificial media.

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EXPLANATION OF PLATES

Plate I.

A The CO_2 absorption apparatus

1. Shell filled with $Ba(OH)_2$.
2. Intake tube from flask.
3. Rubber to hold 5 in place.
4. Outlet from absorption shell.
5. Absorption shell made out of test tube.
6. Rubber connection with detachable tube. This connection may be closed by means of a pinch cock.
7. Detachable tube for collecting $BaCO_2$.
8. Rubber tube to facilitate refilling.
9. T tube connecting units in series, and the series with the suction pump.

B Thermos flask with attachments

1. Intake for CO_2 free air.
2. Outlet for vitiated air connected to A2.
3. Copper-constantin junction.
4. Insulated wires to switch-board.

C Trap containing strong KOH.

The following plates show graphically the average temperatures obtained on each day of observation. They also show the average hourly production of carbon dioxid. All the experiments

were carried out with pure cultures of fungi on alfalfa hay, 40 per cent moisture content.

Plate II

Fig. 1. Aspergillus fumigatus Fres.

Fig. 2. Aspergillus niger Van Tieghem

Fig. 3. Aspergillus flavus Link

Plate III.

Fig. 1. Rhizopus nigricans Ehrenberg

Fig. 2. Aspergillus clavatus Desm.

Fig. 3. Aspergillus terreus Thom

Plate IV.

Fig. 1. Hormodendron nigrescens Paine

Fig. 2. Penicillium oxalicum Currie and Thom

Fig. 3. Penicillium humicola Oud.

Plate V.

Fig. 1. Rhizopus tritici Saito

Fig. 2. Mucor abundans Povah

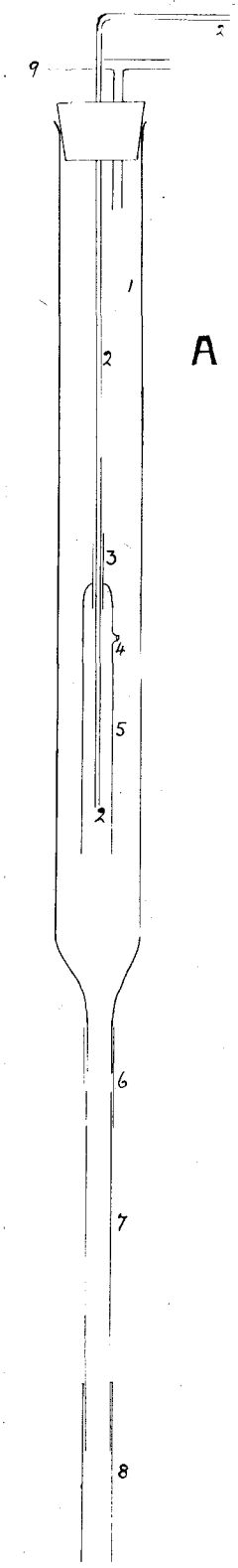
Fig. 3. Cunninghamella elegans Lendner

Plate VI.

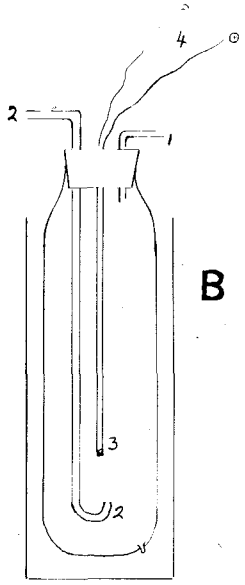
Fig. 1. Spicaria divaricata (Thom) Gilman and Abbott

Fig. 2. Alternaria humicola Oud.

Plate I.



A



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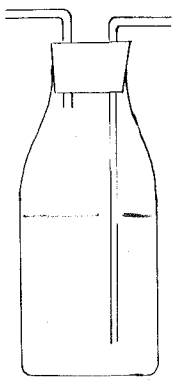


C

Plate II.

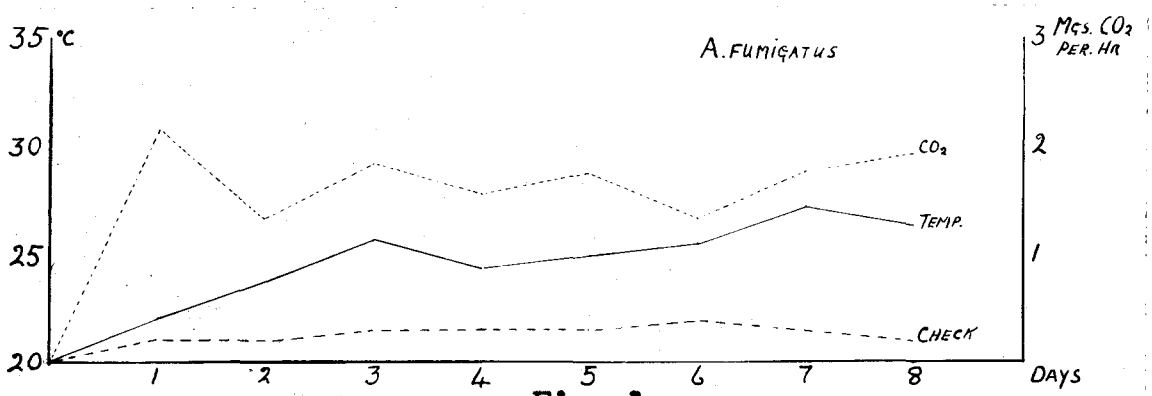


Fig. 1.

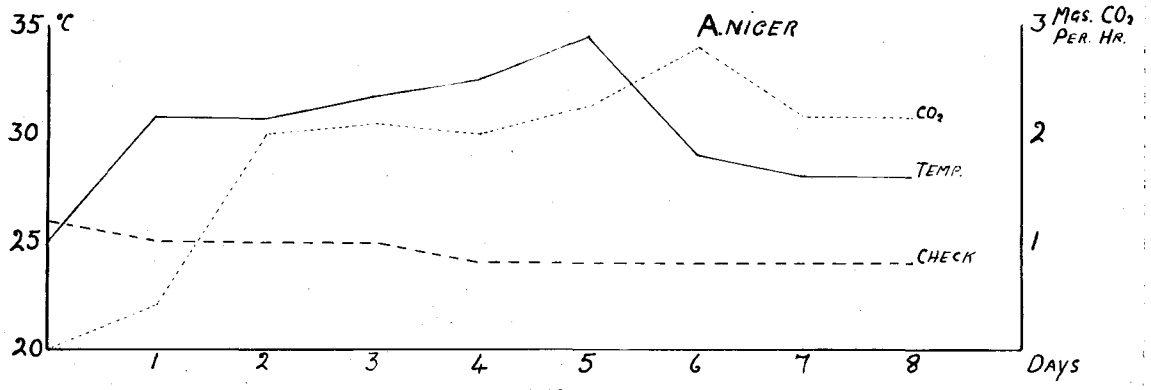


Fig. 2.

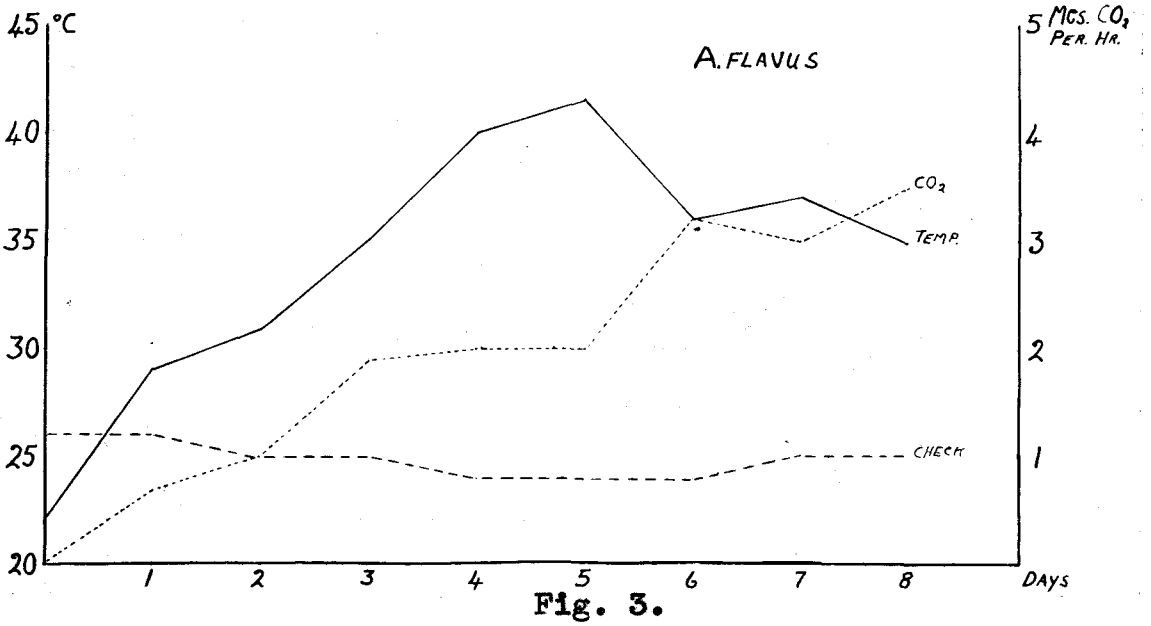
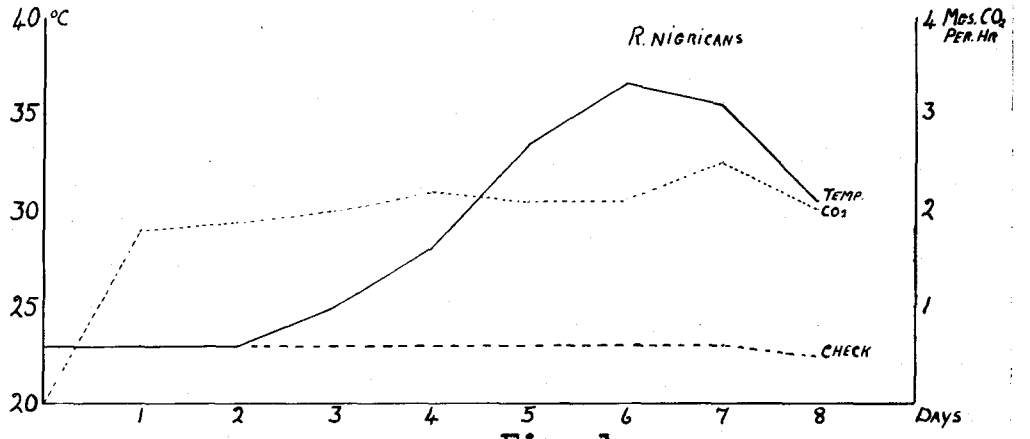


Fig. 3.

Plate III.



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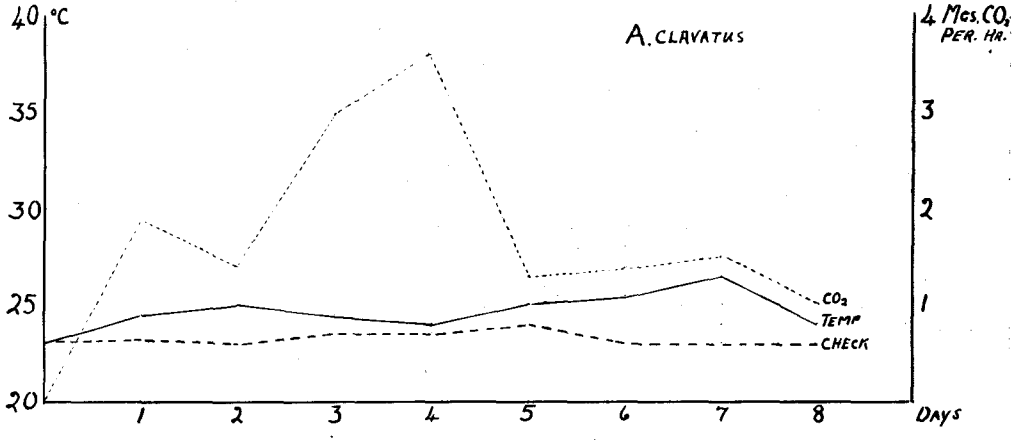


Fig. 2.

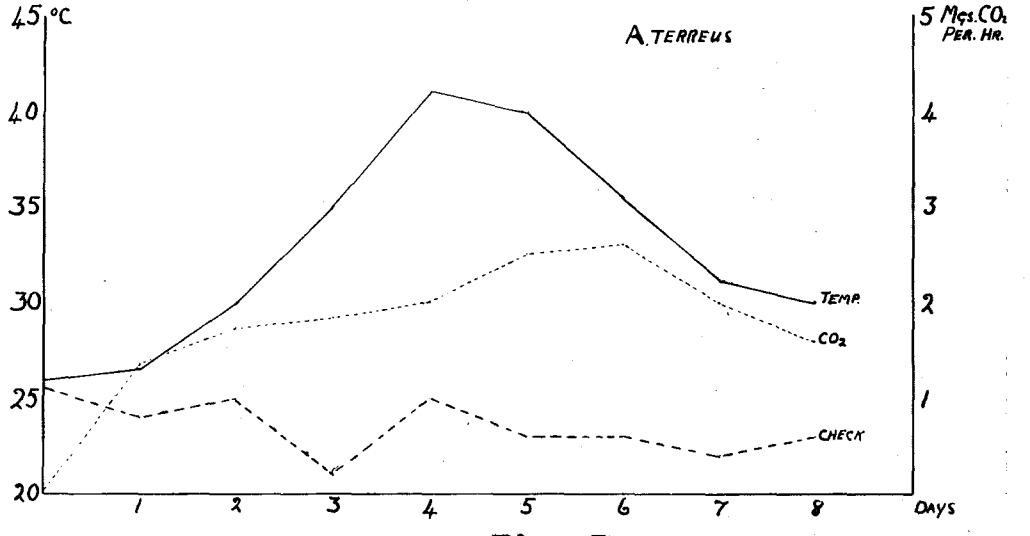


Fig. 3.

Plate IV.

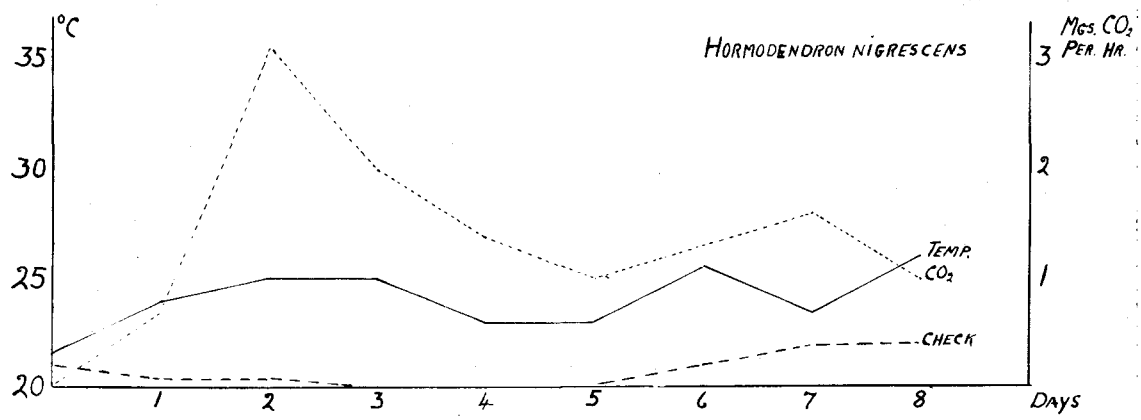


Fig. 1.

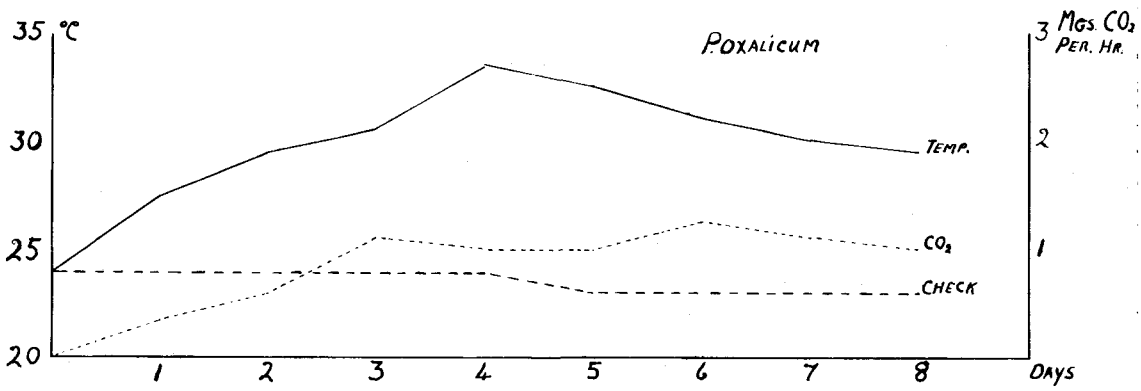


Fig. 2.

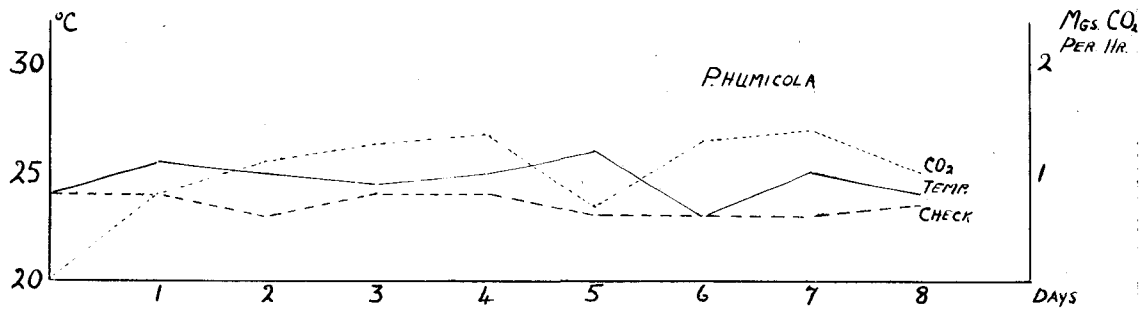
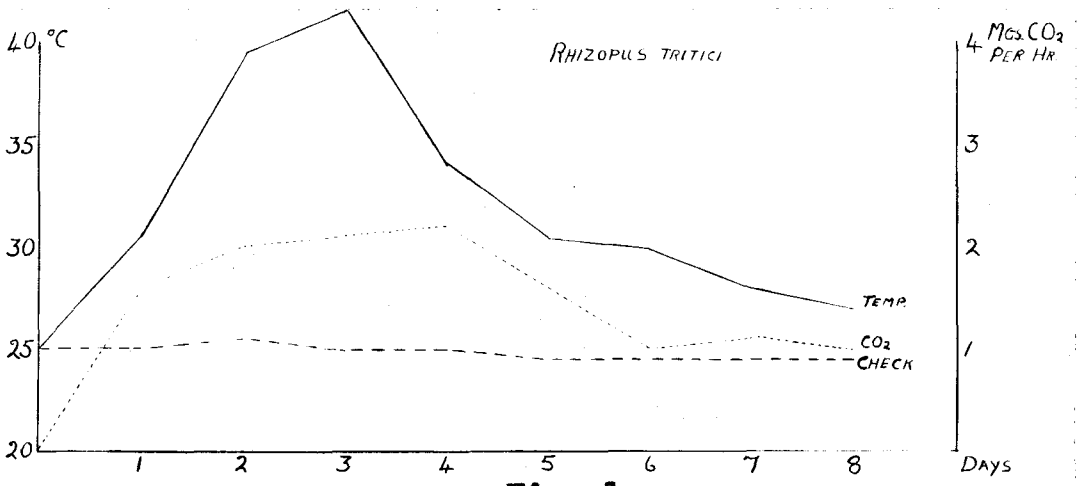


Fig. 3.

Plate V.



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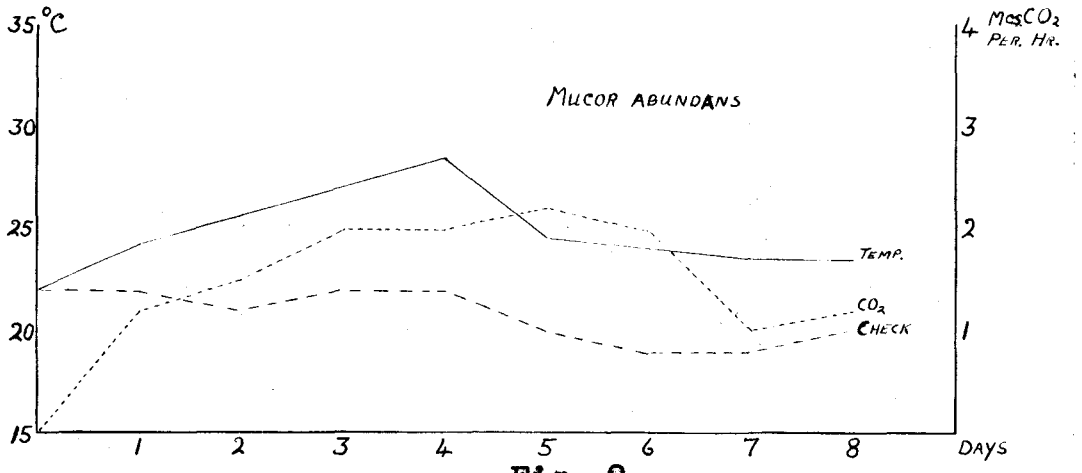

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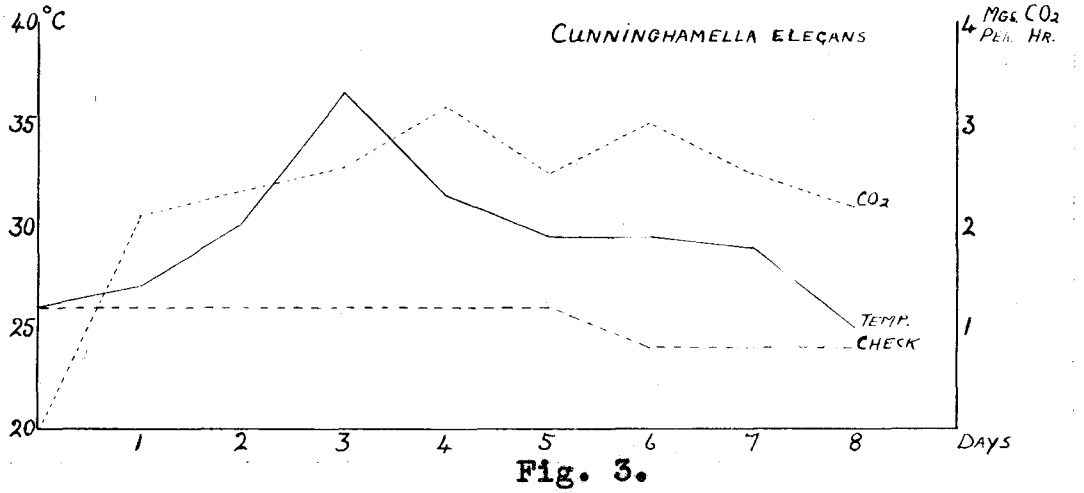

Fig. 3.

Plate VI.

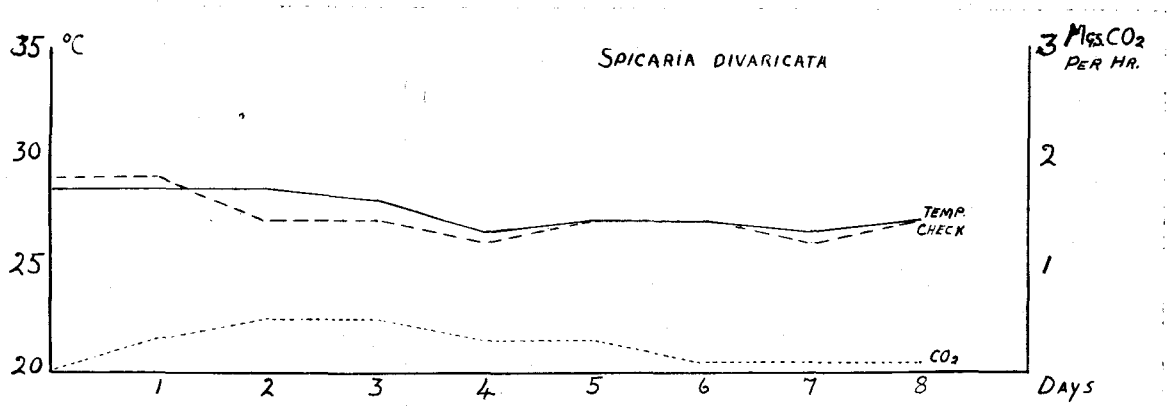

Fig. 1.

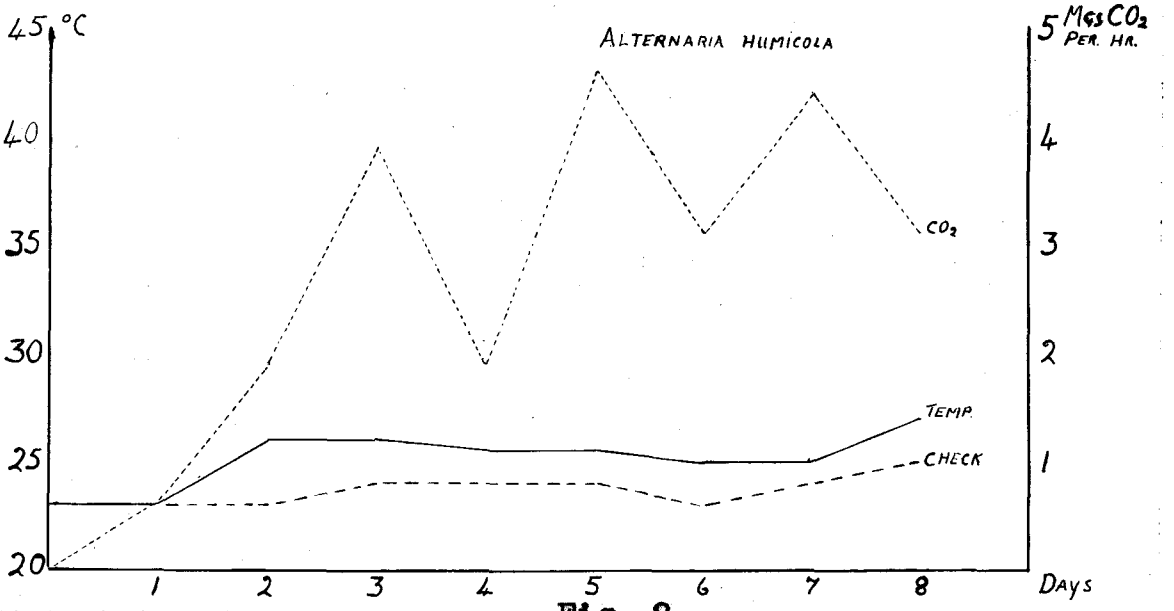

Fig. 2.